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PRINCIPAL INVESTIGATOR: Gary D. Luker, Ph.D.

CONTRACTING ORGANIZATION: The University of Michigan
Ann Arbor, MI 48109

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Year 2 Annual Report: W81XWH-09-1-0128
Imaging CXCL12-CXCR4 Signaling and Inhibition in Ovarian Cancer

1. Introduction. CXCR4 and its chemokine ligand CXCL12 are potential targets for molecular therapy of ovarian cancer. Receptor CXCR4 is expressed by ovarian cancer cells in approximately 50% of patients. High levels of CXCL12 are present in ascites of patients with ovarian cancer, providing a local source of chemokine ligand in the tumor microenvironment. CXCL12 signaling through CXCR4 activates pathways that could promote tumor growth, invasion, metastasis, and resistance to chemotherapy. To develop CXCL12-CXCR4 as molecular targets for chemotherapy in ovarian cancer, there is an unmet clinical need to understand how these signaling pathways function in the tumor microenvironment of ovarian cancer *in vivo*. We propose to develop new molecular imaging technologies and signaling reporters to analyze CXCR4 signaling *in vivo* and determine to what extent inhibiting CXCL12-CXCR4 signaling produces disease regression in mouse models of ovarian cancer.

2. Body.

Aim 1. To construct ovarian cancer cell lines with molecular imaging reporters to quantify activation of CXCR4 and downstream effector molecules.

A. Time period: Months 1-16

B. Tasks to be completed:

1. Establish ovarian cancer cell lines (Hey-8, Skov3i.p.1) stably expressing CXCR4 imaging reporters for receptor homodimerization, recruitment of β -arrestin 2, and activation of AKT.
2. Test responses of each reporter cell line to stimulation with CXCL12 and inhibition with CXCR4 inhibitors AMD3100 and TF1403 in cell-based assays.
3. Test CXCL12-dependent activation of signaling pathways in reporter cells using biochemical assays including Western blotting.

Progress. 1. We have established Hey-8 cell lines with these reporters except for the AKT construct. We have been unable to generate a reporter construct that produces sufficient specific signal to detect and quantify this pathway. As we continue to pursue this objective, we developed an alternative imaging reporter to quantify CXCL12 binding to CXCR4 in cell-based assays and living mice. We will continue to use lentiviral vectors to transfer these reporters to Skov3i.p. cells).

2 – 3. We focused on validating reporters with AMD3100 because this agent now is a clinically-approved drug with potential for direct translation to clinical trials in ovarian cancer. Use of AMD3100 to inhibit reporter signals in HeyA8 cells is shown in Figures 1-2. As described above, work with the AKT reporter has not been completed because of ongoing technical challenges with signal to background. A Western blot documenting CXCL12 activation of CXCR4 signaling in HeyA8 cells is shown in Figure 3.

Aim 2. Image and quantify activation of CXCR4 signaling and pharmacodynamics of CXCR4 inhibitors in mouse models of ovarian cancer.

A. Time period: Months 13-36

B. Tasks to be completed:

1. Establish intraperitoneal ovarian cancer xenografts of Hey-8 or Skov3i.p. cells stably expressing CXCR4 imaging reporters. Xenografts will be established in Ncr^{nu/nu} (nude) mice.

2. Perform bioluminescence imaging and MRI studies of CXCR4 signaling and tumor progression.

3. Quantify inhibition of CXCR4 signaling with known and candidate CXCR4 inhibitors and establish to what extent inhibition of CXCR4 signaling correlates with tumor growth and overall survival.

Progress. 1. We successfully established xenografts of Hey-8 ovarian cancer cells expressing imaging reporters for recruitment of β -arrestin 2 to CXCR4 and a reporter system for imaging CXCL12 binding to CXCR4. We observed improved tumor take for xenografts and subsequent growth of intraperitoneal tumors in severe combined immunodeficiency mice (SCID) lacking the γ subunit of the interleukin 2 receptor (NOG mice). We are using these mice instead of nude mice to improve tumor growth (Figures 4-5).

2. We have performed initial bioluminescence imaging experiments with these two reporters (Figures 5-8). Since initial MRI studies were degraded by motion artifact from intestines and breathing, we developed alternative imaging reporter system based on far red fluorescent protein FP650 to quantify total tumor burden in living mice (Figure 4). We continue to work toward achieving an AKT reporter system that provides sufficient specific signals for imaging in living animals.

3. We treated mice with AMD3100 (plerixafor), a clinically approved small-molecule inhibitor of CXCR4. Imaging reporters showed that treatment with this drug blocked CXCR4 signaling in tumor-bearing mice, as compared with mice receiving vehicle control (Figures 5-8). Treatment with AMD3100 produced a statistically significant improvement in overall survival (Figure 9).

Key Research Accomplishments.

- Established that AMD3100 blocks CXCL12 binding to CXCR4 and recruitment of β -arrestin 2 in cultured cells and a mouse xenograft model
- Found that AMD3100 prolongs survival in a mouse xenograft model of human ovarian cancer

Reportable Outcomes.

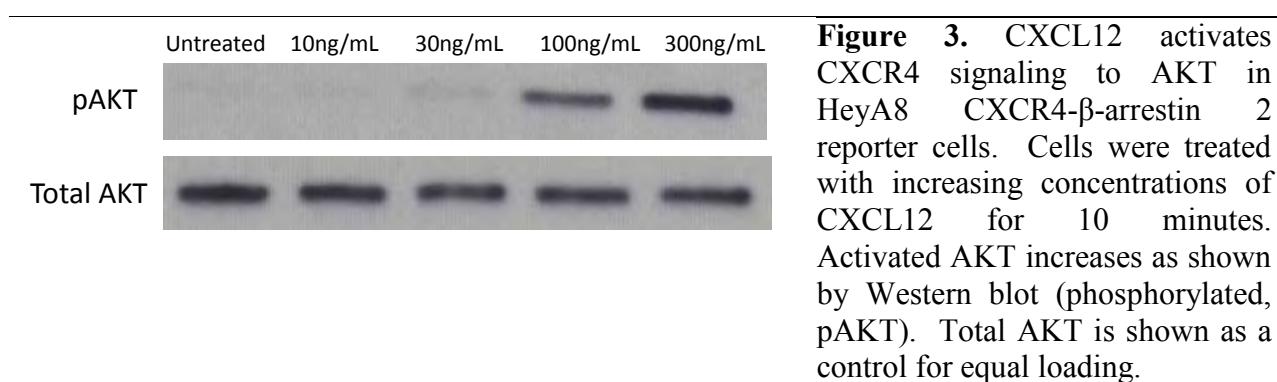
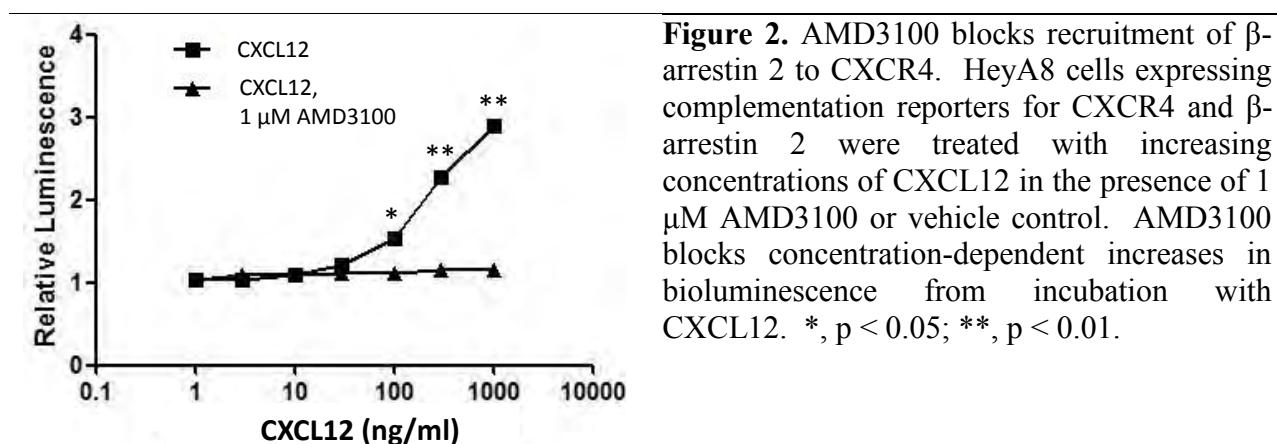
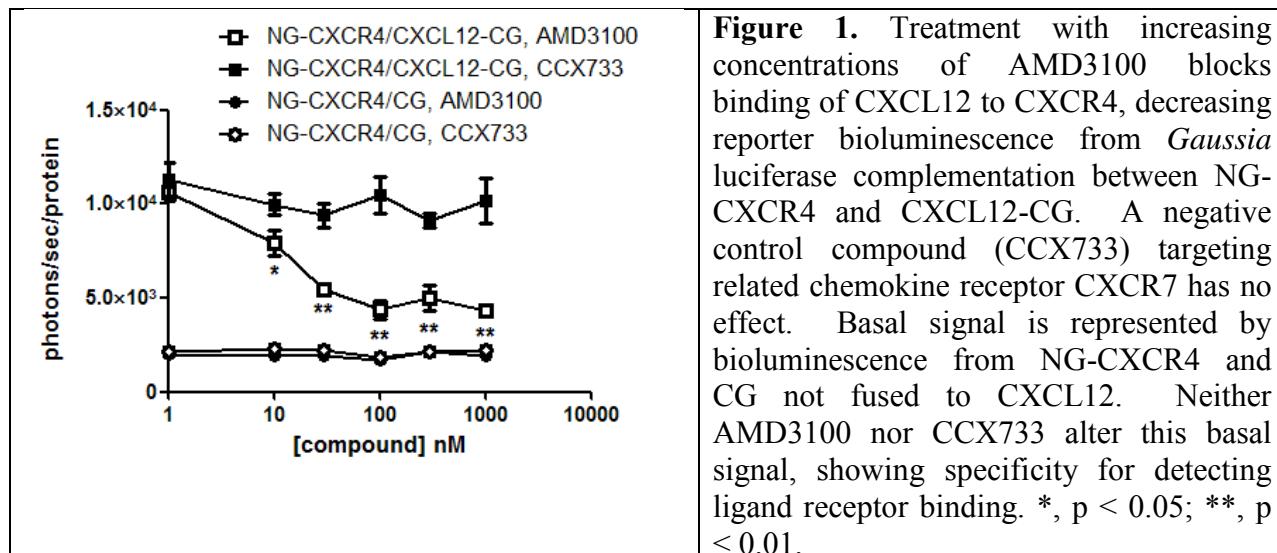
- Validated performance of imaging reporters for CXCL12 binding to CXCR4 and recruitment of β -arrestin 2 in cultured cells and a mouse xenograft model of ovarian cancer

Conclusion. The research to date validated two different molecular imaging reporters for CXCR4 activation and signaling in a mouse xenograft model of human ovarian cancer. These reporters allow pharmacodynamics of targeted therapeutic agents to be quantified in real time in living mice. We also established that treatment with a clinically-approved inhibitor of CXCR4 extends survival in a mouse model of human ovarian cancer.

During the third year of funding, we will continue efforts to develop an AKT reporter for downstream signaling from CXCL12-CXCR4. We also will combine existing luciferase reporters for CXCL12-CXCR4 binding and recruitment of β -arrestin 2 to image sequential steps in the signaling pathway.

Supporting Data:

Aim 1.



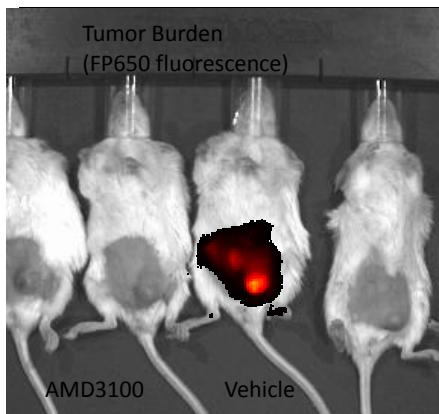


Figure 4. Hey-8 cells expressing fluorescent protein FP650 were injected intraperitoneally. Fluorescence imaging shows total tumor burden in the abdomen for mice treated with either AMD3100 or vehicle control. Scale bar shows range of fluorescence radiance depicted on pseudocolor scale.

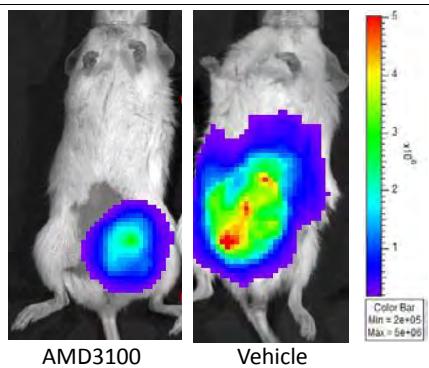


Figure 5. Hey-8 cells expressing luciferase complementation reporters for CXCR4 association with β -arrestin 2 were injected intraperitoneally. Imaging was performed 2 days after starting treatment with 7-day osmotic infusion pumps loaded with 25 mg/ml AMD3100. Scale bar shows range of luciferase photons depicted on pseudocolor scale.

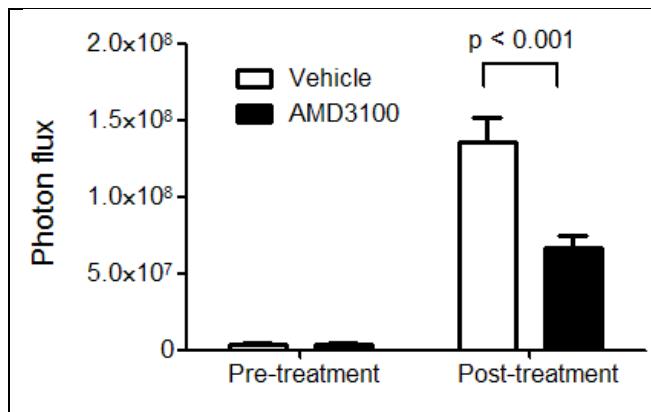


Figure 6. Quantified data from mice ($n = 5$ per group) with CXCR4- β -arrestin 2 complementation reporters shown in figure 5. Data show photon flux values from mice before treatment (pre-treatment) or after 2 days of treatment with AMD3100 (post-treatment). Graph shows mean values + SEM. Treatment with AMD3100 significantly reduced activation of CXCR4, as evidenced by a decrease in photon flux values for the complementation reporter.

